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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No.

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Quinn Q. Patrick Y.	TANG LU	Gaithersburg, MD Rockville, MD

 Additional inventors are being named on the 1 separately numbered sheets attached hereto**TITLE OF THE INVENTION (500 characters max)**

RNAi Therapeutics for Treatment of Eye Neovascularization Diseases

CORRESPONDENCE ADDRESS

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ENCLOSED APPLICATION PARTS (check all that apply) Specification Number of Pages

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 Application Data Sheet. See 37 CFR 1.76**METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT** Applicant claims small entity status. See 37 CFR 1.27. A check or money order is enclosed to cover the filing feesFILING FEE
AMOUNT (\$) The Commissioner is hereby authorized to charge filing
fees or credit any overpayment to Deposit Account Number:

08-1641

80

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

 No. Yes, the name of the U.S. Government agency and the Government contract number are: _____Respectfully submitted,
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2/5/04

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Docket Number:

38147-0026

**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT
PROVISIONAL APPLICATION COVER SHEET**

Additional Page

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Docket Number	38147-0026
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INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Martin C.	WOODLE	Bethesda, MD

Number 2 of 2

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FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

 Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$ 80)

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Application Number	Not yet assigned
Filing Date	February 5, 2004
First Named Inventor	Quinn Q. TANG et al.
Examiner Name	Not yet assigned
Art Unit	Not yet assigned
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METHOD OF PAYMENT (check one)

 Check Credit card Money Order Other None Deposit Account:Deposit Account **08-1641 (Docket No. 38147-0026)**Deposit Account Name **Heller Ehrman White & McAuliffe LLP**

The Commissioner is authorized to: (check all that apply)

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3. ADDITIONAL FEES

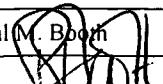
Large Fee Code	Entity Fee (\$)	Small Fee Code	Entity Fee (\$)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for <i>ex parte</i> reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

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SUBTOTAL (3) (\$)

Complete if applicable

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**RNAi Therapeutics for
Treatment of Eye Neovascularization Diseases**

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RNAI THERAPEUTICS FOR TREATMENT OF EYE NEOVASCULARIZATION DISEASES

FIELD OF THE INVENTION

The present invention provides methods and compositions for treatment of eye neovascularization diseases using RNA interference (RNAi) mediated knockdown of pro-angiogenesis genes in mammalian eyes, with a systemically targeted delivery of siRNA duplexes. This innovative approach allows specific siRNA duplexes, designed to knockdown expressions of the pro-angiogenesis genes, such as, VEGF derivatives, VEGF receptors, FGFs and their receptors, and PDGFs and their receptors, to be delivered locally and systemically with, for example, chemically synthesized carriers and ligand-directed chemically synthesized carriers. Using this approach, siRNA-mediated specific antiangiogenic effects are localized to the desired tissues. This approach can be applied with other types of nucleic acids, protein peptides and small molecules to inhibit excessive neovascularization caused by different types of eye diseases. This siRNA-mediated antiangiogenesis therapy is useful, for example, for treatment of external eye infections, diabetic retinopathy, age-related macular degeneration and eye cancer.

BACKGROUND

Ocular neovascularization (NV) is abnormal proliferation of new blood vessels within the eye, is an early pathological step of many eye diseases and is the most common cause of permanent blindness in United States and Europe. There are several major eye diseases and disorders associated with NV. Examples of some of these diseases and disorders are as follows.

Diabetic Retinopathy (DR) is a disorder which occurs when tiny blood vessels which provide oxygen to the retina become damaged. The damage allows blood and fluid to escape into the retina and can also result in new blood vessel growth. These new vessels are fragile and frequently bleed into the vitreous. Patients with the most serious form of DR are at a substantial risk for severe visual loss without treatment.

Age related macular degeneration (AMD) is a leading cause of blindness in people over 60 years of age. In AMD, central vision is lost making it impossible to

appreciate fine detail. In AMD, the retinal pigment epithelium (RPE) plays a pivotal role. Abnormal waste material builds up beneath and within the RPE and eventually RPE cells die. The rods and cones in the retina depend for their survival upon normal functioning RPE and so this RPE failure leads to progressive loss of vision. To make matters worse, the disease provokes a scarring process at the back of the eye with the formation of new blood vessels. The retina is very intolerant to this scarring and again, rods and cones are lost.

Uveitis is an eye autoimmune disease with inflammation of the tissue on the inside of the eye. This disease is most commonly classified anatomically as anterior, intermediate, posterior or diffuse. Ocular complications due to uveitis may produce profound and irreversible loss of vision, especially when unrecognized or treated improperly. The most frequent complications include, for example, cataract; glaucoma; retinal detachment; neovascularization of the retina, optic nerve, or iris.

Rubeosis is abnormal blood vessel growth on the iris and the structures in the front of the eye. Normally, there are no visible blood vessels in these areas. When the retina has been deprived of oxygen, or ischemic, as with diabetic retinopathy or vein occlusion, abnormal vessels form to supply oxygen to the eye. Unfortunately, the formation of these vessels obstructs the drainage of aqueous fluid from the front of the eye, causing eye pressure to become elevated. This usually leads to neovascular glaucoma.

Choroidal Neovascularization (CNV) is the result and cause of many ocular diseases, such as those described above. Moreover, external eye infections, such as, Conjunctivitis, Keratitis, Blepharitis, Sty, Chalazion and Iritis, are also major causes of ocular neovascularization.

Herpes Simplex Virus (HSV) infection (and recurrent HSV infection) is a common infectious cause of corneal blindness in the U.S. The viral infection causes blinding lesions called stromal keratitis (SK). The corneal NV is the early step leading to the herpetic SK. HSV infection stimulates the over-expression of vascular endothelial growth factor (VEGF) that results in the development of ocular NV. Therefore, the VEGF pathway of the angiogenesis is likely an effective area for developing treatments to this disease.

As National Eye Institute of NIH has estimated, 400,000 Americans have had some form of ocular herpes, and there are nearly 50,000 new and recurring cases diagnosed each year in the United States, with the more serious stromal keratitis accounting for about 25%. From a larger study, it was found that the recurrence rate of ocular herpes is 10 percent in one year, 23 percent in two years, and 63 percent within 20 years. Although application of available anti-viral drugs could control the HSV infection to some extent, there is a need for a more effective treatment.

VEGF is an essential growth factor responsible for normal vasculogenesis and angiogenic remodeling (1, 2, 3). Under some disease conditions, the VEGF angiogenic pathway will be activated, such as in the situation of tumors where new blood vessels are formed to deliver enough oxygen and nutrition to the rapidly growing abnormal tissues. The majority of severe visual loss in the United States results from complications associated with retinal neovascularization in patients with ischemic ocular disease such as diabetic retinopathy, retinal vein occlusion, and retinopathy of prematurity. Intraocular expression of the angiogenic protein VEGF is closely correlated with neovascularization in these human disorders and with ischemia-induced retinal neovascularization in mice. Therefore, the VEGF pathway composed, for example, of VEGFs and VEGF receptors, is a logical target for inhibition of retinal angiogenesis. Using siRNA to knockdown the expressions of, for example, the pro-angiogenesis genes: VEGF A, VEGF R1, and VEGF R2, represents a novel specific therapy for ischemic retinal disease, and other related eye diseases.

RNAi techniques have been developed, for example, in cell culture and in model organisms such as *Drosophila*, *C. elegans*, and zebrafish. Studies of RNAi have found that long dsRNA is processed by Dicer, a cellular ribonuclease III, to generate duplexes of about 21 nucleotides (nts) with 3'-overhangs, called short interfering RNA duplexes (siRNA), which mediate sequence-specific mRNA degradation (4, 5, 6). Use of siRNA to interfere with expression of a specific gene is preferably accompanied by knowledge of target accessibility, effective delivery of the siRNA into the target cells, and, for some biological applications, long-term activity of the siRNA in the cell. siRNA can be used, for example, as a functional genomic tool and as novel therapeutics. Therapeutic applications will clearly include optimized local and systemic delivery methods. The

advantages of using siRNA as a therapeutic agent are, for example, due to its specificity (7, 8), stability (9) and mechanism of action (4, 5, 6).

We have used siRNA to silence pro-angiogenic factors in tumor models and have demonstrated strong silencing effects (10). This progress exhibited the feasibility and efficacy of RNAi silencing of pro-angiogenic factors *in vitro* and *in vivo*, and proved that our molecular design and proprietary delivery system are applicable for siRNA-mediated gene silencing to treat ocular neovascularization.

With the objective of using anti-angiogenic RNAi for the development of novel therapeutics for ocular NV diseases, we used the published animal model of corneal NV induced by CpG previously implanted in the micropocket in mice cornea stroma, and through which the inhibition of angiogenesis can easily be measured. We have designed a series of siRNAs duplexes targeting messenger RNAs (mRNAs) of mVEGF, mVEGF-R1, and mVEGF-R2, with two target sequences per mRNA. The knockdown effects of these siRNA molecules were first tested in cell culture. Then the selected siRNA duplexes were delivered into the mouse model described above. Two delivery systems were applied.

A systemic delivery system, developed in house and named as TargeTran™ was used for the siRNA delivery (11). A delivery carrier that can be used is a cationic polymer based technology that has been disclosed by Woodle *et al.* (WO 01/49324, filed December 28, 2000). As used herein, a “synthetic vector” means a multi-functional synthetic vector which, in one embodiment, at a minimum, contains a nucleic acid binding domain and a ligand binding (*e.g.* tissue targeting) domain, and is complexed with a nucleic acid sequence. A synthetic vector also may contain other domains such as, for example, a hydrophilic polymer domain, endosome disruption or dissociation domain, nuclear targeting domain, and nucleic acid condensing domain. A synthetic vector for use in the present invention preferably provides reduced non-specific interactions, yet effectively can engage in ligand-mediated (*i.e.* specific) cellular binding. In addition, a synthetic vector for use in the present invention is able to be complexed to one or more therapeutic nucleic acids, which then can be administered to a subject. We have administrated these siRNAs with this systemic approach by tail vein injection. The

knockdown effects for VEGF A, VEGF R1 and VEGF R2 resulted in significant inhibition of ocular neovascularization (13).

We also have used another polymer-based carrier, PolyTranTM, to deliver siRNAs in vitro and in vivo. This preparatory technology (WO 0147496, Histidine copolymers and methods for using same) is able to substantially reduce the formation of neovasculature induced by CpG in the otherwise avascular mice eye cornea. Figures 5-7 show successful design and use of siRNA with applications for treating herpetic SK and other angiogenic eye diseases such as those discussed above.

Rationale

The normal cornea is avascular, and HSV does not make angiogenic proteins, therefore there must be angiogenic factors expressed in the infected eyes stimulated by HSV infection that induces corneal NV. It was demonstrated that HSV eye infection stimulated the expression of VEGF, and VEGF played an important role in the ocular herpetic NV. VEGF mRNA became detectable twelve hours after HSV infection (p.i.); the VEGF protein became detectable 12 hours later. The VEGF production occurs initially in the virus-infected corneal epithelial non-inflammatory cells, but later in the clinical phase, inflammatory cells (PMNs and macrophages) in the stroma became the only cell population responsible for VEGF production. In a mice eye model, corneal NV was experimentally induced by purified HSV viral DNA (HSV DNA, rich in CpG motifs) or the synthesized CpG oligonucleotide (CpG ODN). This model created a simple way to induce corneal NV, and is ideal for the testing antagonists of VEGF or its receptors in their efficacy in inhibition of angiogenesis.

RNAi, the double stranded RNA (dsRNA)- induced sequence-specific degradation of messenger RNA (mRNA), or called gene silencing, has been proven to be a powerful tool for gene discovery or gene validation, and it holds great potential in developing novel gene-specific drugs. In our anti-angiogenic RNAi design for the inhibition of eye NV, mVEGF-A, mVEGF-R1, and mVEGF-R2 are chosen to be the target genes that are key players in the VEGF angiogenic pathway. Small interfering RNAs (siRNAs) are designed according to general guideline proposed by Tuchl's research team. They are 21-nucleotide long double stranded RNAs with 2-nt overhangs at

either 3' termini, with the negative strand complementary to the targeted mRNA sequences. The knockdown of these genes, singly or in combination, has the impact of blocking the angiogenic pathway leading to the inhibition of NV, and thus the relief of the SK symptoms. The same scenario will apply to other NV-related ocular diseases.

Fields of the Invention

The present invention provides a novel molecular strategy of using RNAi to target and silence the pro-angiogenic genes in animal, including humans and mammals in particular, eyes, and providing great potential to develop new therapeutics to treat ocular neovascularization diseases caused by, for example, Diabetic Retinopathy (DR), Age related macular degeneration (AMD), an eye autoimmune disease Uveitis, stromal keratitis (SK) and eye cancers. The present invention provides molecular design of the therapeutic molecules, siRNA duplexes, their effectiveness for knocking down VEGF A, VEGF R1 and VEGF R2 both in vitro and in vivo using, for example, the delivery systems described herein. The present invention also provides a novel and clinical feasible delivery of siRNA agent for treatment of neovascularization which is the causes of many inflammatory diseases and cancers.

Summary of Invention:

The present invention provides interfering small double stranded RNAs and delivery systems to knockdown the expression of pro-angiogenic factors in animal eyes. The invention includes: 1) using RNAi to treat NV-related eye diseases; 2) using a clinically relevant ocular NV mice model to test RNAi effect *in vivo*, where CpG ODN is used to induce corneal NV; 3) using special pro-angiogenic genes as targets; 4) the design of interfering RNAs, including the sequences, the length and overhangs of these molecules; 5) using the polymer system (TargeTranTM) for tissue-specific targeting; 6) using the polymer system (PolyTranTM) for siRNA delivery; 7) using non-invasive systemic delivery of siRNA into mice; 8) using local and topical delivery of siRNA into eyes; 9) using methods and reagents for RNA template-specific RT-PCR for detecting the knockdown of the targeted mRNA synthesis; 10) using an experimental platform able to test other forms of interfering RNAs, e.g., dsRNA, shRNA, and ddRNAs; and 11) a

design useful in treating ocular NV-related eye diseases which is useful in the treatment of animals, for example, humans and mammals.

Detailed Description of the Invention:

I. Using RNAi to treat VN-related ocular diseases

Ocular NV is involved, often at an early stage, in a variety of eye diseases including HSV-incurred corneal stroma ketatitis (herptic SK) and the related blindness, and eye cancers. There is a lack of effective therapeutic applications for ocular NV in clinical practice. RNAi presents a novel potent strategy to knock down target genes by destroying the mRNAs in a sequence-specific manner. The RNAi effect can modulate be biologically function instantaneously, or over a long and sustained period of time. The present invention includes novel molecular compositions and methods for treating corneal NV or other NV-related eye diseases.

II. Using a clinically relevant ocular NV mice model to test RNAi effect *in vivo*

A recent mouse eye model presents a feasible and clinically relevant model for corneal NV. In this model, purified HSV DNA (CpG rich) and/or synthetic CpG motif-oligonucleotide (CpG ODN) are used to induce VEGF expression in cornea, thus inducing NV, and corneal SK, instead of using HSV infection or VEGF proteins. In this model, the new blood vessel formation is easily induced and measured. The present invention employs, for example, this model to test the interfering RNAs and to collect data on the RNAi therapy of the CpG-induced SK. Using this model, experimentation, handling and quantitative analysis are relatively easy and the model is predictive of HSV infection-related eye SK in human. These characteristics make our *in vivo* RNAi data extremely useful for evaluating RNAi-based therapeutics for the treatment of HSV infection-caused SK and other angiogenic eye diseases.

III. The pro-angiogenic genes to be targeted.

The VEGF family is composed of five structurally related members: VEGF-A, Placenta Growth factor (PIGF), VEGF-B, VEGF-C, and VEGF-D. There are three structurally homologous tyrosine kinase receptors in the VEGF receptor family: VEGFR-1 (Flt-1), VEGFR-2 (KDR or Flk-1), and VEGFR-3 (Flt-4), with different affinity or functions related to different VEGF members. VEGF-A, which binds VEGFR-1 and VEGFR-2, is known to induce neovascularization, angiogenesis, and vascular permeability. In order to functionally interact with their specific receptors VEGF naturally form homo-dimers.

VEGFR-1 and VEGFR-2 both are up-regulated in tumor and proliferating endothelium that may be a direct response to VEGF-A or partly to hypoxia. VEGFR-2 mediates angiogenic signals for blood vessel growth, and is necessary for proliferation. VEGFR-1 has higher affinity to VEGF-A than VEGFR-2, and mediates motility and permeability, and therefore may play a role in transducing angiogenic signals. However, some observations suggested that VEGFR-1 may act as a decoy receptor for VEGF/VEGFR-2 signaling. The understanding of basic biology of the VEGF and VEGFR receptors provides solid foundation for the design of approaches to target the VEGF signaling pathway. In our RNAi approach, one mVEGF (mVEGF-A) and two mVEGFR receptors (mVEGFR-1 & mVEGFR-2) are targeted by interfering RNAs.

IV. Design of interfering RNAs

By DNA comparison with Clone Manager Suite and by on-line Blast search, the targeted sequences of mVEGF-A are confirmed unique for mVEGF-A, that will not target mVEGF-B mVEGF-C, mVEGF-D, nor the human counterpart, hVEGF165-a (AF486837). However, they will target some isoforms of mVEGF-A, e.g., mVEGF (M95200), mVEGF115 (U502791), mVEGF-2 (S38100), mVEGF-A (NM_192823), that encode mVEGF-A proteins of 190 amino acid (aa), 141 aa, 146 aa, and 148 aa, respectively. All of the published cDNA sequences of these mVEGF-A isoforms, except mVEGF-A (NM_192823, a mature form of protein), include a 26-aa signal peptide at N'-terminus. The targeted sequences of mVEGF are chosen not in the signal peptide part, but

in the mature protein part shared by all these mVEGF-A isoforms. Targeted sequences of mVEGF-R1 and mVEGF-R2 are also confirmed unique for these two genes, respectively. Different forms of interfering RNAs are included in present invention. As an example, the small interfering RNAs, siRNAs, are designed according to the above target sequences, and basically follow the guidelines accepted by RNAi researchers worldwide. These siRNAs are 21-nt double stranded RNA oligos with 2 nts (TT) at 3' overhangs. The targeted sequences (mRNA sequences) and the sequences of siRNAs are listed in Table 1.

Table 1. The targeted genes and the targeted mRNA sequences

Genes	Targeted sequences (5'-3')		Notes
mVEGF-A	1	AAGCCGTCCTGTGTGCCGCTG	91-111 nt of cds, or 151-171 or XM_192823 sequence.
	2	AACGATGAAGCCCTGGAGTGC	133-153 nt of cds, or 193-213 nt of XM_192823 sequence.
mVEGFR-1	1	AAGTTAAAAGTGCCTGAAGTG	82-102 nt of cds, or 333-353 nt of D88689
	2	AAGCAGGCCAGACTCTCTTTC	131-151 nt of cds, or 382-403 nt of D88689
MVEGFR-2	1	AAGCTCAGCACACAGAAAGAC	97-117 nt of cds, or 304-324 nt of NM_010612
	2	AATGCGCGGGTGGTGACAGTA	233-243 nt of cds, or 440-460 nt of NM_010612

V. Polymer system (TargeTranTM) used for tissue-specific targeting.

An example of a delivery system is TargeTranTM which consists of three functional domains: a cationic poly-ethylneimine (PEI), a polyethylene glycol (PEG), and a RGD-peptide. The PEI domain condenses the nucleic acid (DNA or RNA) as employed in routine transfection of cultured mammalian cells. The PEG domain protects nucleic acids being delivered from degradation, it also shields the surface charge thus preventing non-

specific charge-mediated interactions between the nucleic acid and proteins on cellular surface or existing in blood stream. These non-specific interactions usually will result in the all-around distribution of the interested molecules if only cationic polymer is used in the system. The RGD peptide, the third domain of our system, is responsible for tissue-specific targeting to the cell surface integrins that are upregulated in tissues where new blood vessels are formed. This delivery is applied for systemic siRNA delivery when given systemically but to target angiogenic eyes.

VI. The polymer system (PolyTranTM) used for siRNA delivery

The PolyTranTM system, including the first and second generation systems, may also be used. The systems comprise cationic peptides. The second generation PolyTran is specially formulated for the effective delivery of siRNA into cells and tissues. The formula provides protection of siRNA from fast degradation and facilitates the transfection of cells by siRNA. By delivering siRNA with PolyTran, we are able to knock down the expression of endogenous target gene, e.g., LacZ, for up to 80% in *in vitro* experiments. This system is specially designed for local delivery of siRNA into animal eyes.

VII. The non-invasive systemic delivery of siRNA into mice

By use of TargeTran delivery system, we are able to deliver siRNA non-invasively into mice by I.V. tail injection or other ways such as transdermal delivery. This feature is useful for RNAi application in patients of ocular NV-related diseases.

VIII. Local or topic delivery of siRNA into different locations in the eyes.

By the use, for example, of the second generation of PolyTran, we are able to locally deliver siRNAs into eyes for use with, for example, intrastromal infection, corneal micropocketing, and intra-subjunctival injection. Although more invasive than systemic delivery, the local delivery of siRNA may still be desirable in certain situations, e.g., in severe NV conditions or in fast growing tumors. Our invention extends to the incorporation of agents that increase the permeability of corneal epitheliums. This

combination revolutionarily switches the local injection into topical application of siRNAs in the form of eye-drops.

IX. RNA template-specific RT-PCR

1) RNA-specific PCR (RS-PCR)

RS-PCR is used to detect the reduction of mRNA synthesis of the targeted genes. RS-PCR included two consecutive reactions: RNA-specific reverse transcription (RT) and PCR. The total cellular RNA transcript was isolated from the transfected mammalian cells or tissues removed from the tested animals, using RNAwiz (Ambion), and treated with DNase in the way described in Promega's T7RiboMAX manual. The DNA-free RNA was used as template for RT reaction to synthesize the first strand of mRNA-specific cDNA molecules. A mRNA-specific primer designed for the RT contained, from 5' to 3' direction, a special 30 nt sequence, which was not complementary to the targeted gene coding sequence, followed by a 14 nt sequence complementary to part of the coding sequence at around 400 nt 3' to the AUG codon. The RT reaction was performed using MuLv retrotranscriptase (Applied Biosystems) in a volume of 20 μ l. The reaction was performed at 37 $^{\circ}$ C for 30' followed by 42 $^{\circ}$ C 15' and then heated at 94 $^{\circ}$ C for 5'. PCR reaction was performed using GeneAmp kit (PE Biosystems). Each pair of primers used for PCR included the forward primer, which was complementary to the coding sequence starting at about 10 nt 3' to the AUG codon, and the reverse primer, which only has the special 30 nt sequence described above. The 50 μ l reaction contained 1 μ l of 20 μ M stocks of each of the pair of primers, 5 μ l 10x PCRII buffer, 3 μ l 2.5mM MgCl₂, 1 μ l 10mM dNTPs, and 0.5 μ l (5 μ l/ μ l) TaqDNA polymerase. The PCR reaction was performed at 94 $^{\circ}$ C 2', then 35 cycles of 94 $^{\circ}$ C 1'-72 $^{\circ}$ C 2' two-step reaction, followed by 72 $^{\circ}$ C 10' and soaked at 4 $^{\circ}$ C. The samples of the reaction were detected by agarose gel electrophoresis along with DNA size standards. The density of DNA fragment of reasonable size (around 400 bp) reflected the starting level of target gene-specific mRNAs in the transfected cells. The siRNAs are synthesised by Dharmacon and primers synthesized by Elim Biopharmaceuticals.

2) Design of the RS-PCR primers

For mVEGF-A (reference sequence: XM_192823)

Primer 1: mVEGF-A Up (30-mer, 4-33 nt of mVEGF-A coding sequence, or 64-93 nt of cloning sequence).

5'---GAT GTC TAC CAG CGA AGC TAC TGC CGT CCG---3'

Primer 2: mVEGF-A Dn (47-mer, the first 30 is the same as "TS1 primer", the following 17-mer is complementary to the 403-387 nt of mVEGF-A coding sequence, or 463-447 nt of cloning sequence).

5'---GAA CAT CGA TGA CAA GCT TAG GTA TCG ATA caa gct gcc tcg cct tg ---3'

For mVEGFR-1 (reference sequence: D88689)

Primer 3: mVEGFR-1 Up (30-mer, 4-33 bp of mVEGFR-1 coding sequence, or 255-284 of cloning sequence)

5'--- GTC AGC TGC TGG GAC ACC GCG GTC TTG CCT ---3'

Primer 4: mVEGFR-1 Dn (47-mer, the first 30 is the same as "TS1 primer", the following 17-mer is complementary to the 377-361 nt of mVEGFR-1 coding sequence, or 628-612 nt of cloning sequence).

5'---GAA CAT CGA TGA CAA GCT TAG GTA TCG ATA tag att gaa gat tcc gc---3'

For mVEGFR-2 (reference sequence: D88689)

Primer 5: mVEGFR2/400Dn (47-mer, 3' 17-mer complementary to mVEGFR2 400-384 nt)

5'--- GAA CAT CGA TGA CAA GCT TAG GTA TCG ATA ggt cac tga cag agg cg---3'

Primer 6: mVEGFR2/12up (30 -mer, 12-41 of mVEGFR2)

5'---GGC GCT GCT AGC TGT CGC TCT GTG GTT CTG---3'

Table 2. RS-PCR targeted genes and the size of products

#	Target genes	Primers	Size of RS-PCR products
1	mVEGF-A	1&2	400 bp
2	mVEGFR-1	3&4	374 bp
3	MVEGFR-2	5&6	389 bp

X. The experimental platform is able to test other forms of interfering RNAs

Since our TargeTran facilitates the delivery of nucleic acids, and our secondary generation of PolyTran facilitates the local/topic delivery of interfering RNAs, our present invention provides an experimental platform to test many forms of interfering RNA molecules in knocking down pro-angiogenic genes in eyes. These include double stranded RNA (dsRNA), small-hairpin RNA (shRNA), and DNA-derived RNA (ddRNA), etc.

XI. The total design able to be used for other ocular NV-related eye diseases.

XII. The RNAi efficacy able to be translated into human clinical trials

References:

Literature Cited

1. Folkman, J, 1971: Tumor Angiogenesis: Therapeutic Implications. *N. Engl. J. Med.*, Vol. 285, p1182-1186.
2. Leung, D.W., Cachianes, G., Kuang, W.J., Goeddel, D.V., and Ferrara, N., 1989: Vascular Endothelial Growth Factor is a Secreted Angiogenic Mitogen. *Science*, Vol. 246, p1306-1309.

3. Aiello, L.P. et al. 1995: Suppression of Retinal Neovascularization in vivo by Inhibition of Vascular Endothelial Growth Factor (VEGF) Using Soluble VEGF-Receptor Chimeric Proteins. *PNAS*, Vol. 92, pp. 10457-10461.
4. Elbashir, S.M., Lendeckel, W. and Tuschl, T. 2001: RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev.* 15(2):188-200.
5. McManus, M.T. and Sharp, P.A. 2002: Gene Silencing in Mammals by Small Interfering RNAs. *Nature Reviews, Genetics*. Vol. 3. p737-747.
6. Lu, Patrick Y., Frank Y. Xie and Martin Woodle, (2003): SiRNA-Mediated Antitumorigenesis For Drug Target Validation And Therapeutics. *Current Opinion in Molecular Therapeutics*, 5(3):225-234.
7. Chi, Jen-Tsan, H. Y. Chang, N. N. Wang, D. S. Chang, N. Dunphy and P.O. Brown, 2003: Genomewide View of Gene Silencing by Small Interfering RNAs. *PNAS*. 1037853100.
8. Semizarov, D., L. Frost, A. Sarthy, P. Kroeger, D. Halbert and S. W. Fesik, 2003: Specificity of Short Interfering RNA Determined Through Gene Expression Signatures. *PNAS*. 1131959100.
9. Hough, S.R., K.A. Wiederholt, A.C. Burrier, T.M. Woolf and M.F. Taylor, 2003: Why RNAi Makes Sense. *Nature Biotechnology*, 21(7) 731-732.
10. Lu, Patrick, Frank Xie, Quinn Tang, Jun Xu, Puthupparampil Scaria, Qin Zhou and Martin Woodle, 2002: Tumor Inhibition By RNAi-Mediated VEGF and VEGFR2 Down Regulation in Xenograft Models. *Cancer Gene Therapy*, Vol.10, Supplement 1, 011.
11. Lu, P. Y., Scaria, P., Woodle, M. C., Xie F. Y., Xu, J. 2001: Therapeutic Methods For Nucleic Acid Delivery Vehicles. *U.S. patent application* No. 60/330,909
12. Xie, F.Y. Lu, P.Y. et al. 2002: Methods of In Vivo Down Regulation of Targeted Gene Expression By Introduction of RNA Interference. *U.S. patent application*, 60/401,029
13. Mei Zheng*, Dennis M. Klinman†, Małgorzata Gieryńska*, and Barry T. Rouse, 2002: DNA containing CpG motifs induces angiogenesis. *PNAS*, Vol. 99(13).

Example 1. The local siRNA delivery to treat ocular Neovascularizaiton

In the grant application, several possible ocular delivery routes were proposed. We (consultant, Dr. Barry Rouse and the research teams from both parties) believe that intrastromal and subconjunctival routes have the best chance of success for siRNA delivery to achieve NV inhibition in corneal area. Direct delivery of siRNA into corneal stroma, where inflammatory cells (PMNs and macrophages) over-express VEGF protein that is the major cause of the disease, was suggested as the ideal route. However, in the initial study, we found that this intrastromal delivery of siRNA agent has certain limitation in the mice NV model we used. For example, when the angiogenic inducing agent and the siRNA duplexes were delivered separately by injecting the eye surface twice with syringe needles, the siRNA solution injected through the second hole was leaked out through the first hole which was used for deposition of the NV-inducing agent (CpG ODN "pellet"). To overcome this hurdle, we switched to the subconjunctival route. Using this route not only avoided the siRNA leakiness, but also delivered siRNAs close to the limbal area where the new blood vessel formation starts. Our preliminary data demonstrated that local delivery of PolyTran™-mixed siRNA through subconjunctival route is capable of achieving substantial inhibition of ocular NV (Fig. 1). In this experiment, 10 µg siRNA targeting mVEGFA, mVEGFR1 and mVEGFR2 was used for each injection, and NV inhibition was measured on day 4 post-pocketing. As expected, each pair of siRNA duplexes has showed the significant inhibition effect, while a stronger inhibition was observed when combination of all siRNA duplexes targeting the three genes was used at the same siRNA dosage. Clearly, using our proprietary PolyTran™-mediated local delivery of siRNA is able to achieve efficient anti-angiogenesis effect for treatment of ocular neovascularization cause by viral DNA sequences. The control group mice showing no visible toxicity effect also indicated that the PolyTran™-siRNA complex was safe for potential clinical application. The anti-angiogenesis effect lasted for more than 4 days after single siRNA local delivery.

Example 2. The systemic siRNA delivery to treat ocular Neovascularizaiton

As we testing the local delivery with the mouse ocular NV model, a systemic delivery system for IV injection was developed and we called it as TargeTran™. TargeTran™ consists of three functional domains: a cationic poly-ethyleneimine (PEI), a polyethylene glycol (PEG), and an RGD-peptide. The PEI domain condenses the nucleic acid (DNA or RNA) as employed in routine transfection of cultured mammalian cells. The PEG domain protects nucleic acids being delivered from degradation, it also shields the surface charge thus preventing non-specific charge-mediated interactions between the nucleic acid and proteins on cellular surface or existing in blood stream. These non-specific interactions usually will result in the all-around distribution of the interested molecules if only cationic polymer is used in the system. The RGD peptide, the third domain of our system, is responsible for tissue-specific targeting to the cell surface integrins that are upregulated in tissues where new blood vessels are formed. The detail chemistry and application of this system has been described in the PCT filing (see list below). This "self-assembled, ligand-targeted, steric polymer grafted nanoplexes" system is applied in the present mouse study when siRNAs were given systemically but

to target angiogenic eyes. We tested this delivery system using the same siRNA duplexes with the same mouse model as with PolyTran™. The TargeTran™ system was not put into the original SBIR application, simply because we were still in a process of testing the tissue-specificity and efficacy of this novel system. Now, we have already obtained the data to demonstrate the efficacy of TargeTran™ in delivering siRNA to reduce NV in the mouse model. In these preliminary experiments, a single dosage of 40 µg siRNA per mouse was used, and NV reduction was measured and photographed. Figures 2 has illustrated that anti-angiogenic effects of TargeTran™-siRNA nanoparticles 4 days after single dose of IV administration. A quantitative analysis is also showed in Figure 3. To evaluate the duration of siRNA-mediated anti-angiogenesis effect, we also measure the neovasculature area again on day 7 (Fig. 4). The significant NV inhibition was observed even on the 7th day after the deposition of NV inducing agent, comparing to the control group. In this study the cohort size was 10 eyes from 5 mice.

One of major concerns have been expressed repeatedly by the critiques was the stability of siRNA agent in vivo. We have observed in several cell culture studies that the siRNA duplexes were very stable in target tissue and cytoplasm for up to at least 3 days, e.g. xenograft tumor, 293 cells and FRhK-4 cells (data not shown). The duration of those siRNA duplexes were measured with detections of both fluorescence labeling and biological function. As we presented in the data from both local and systemic deliveries, the anti-angiogenesis activity was lasted for more than 4 days. The next challenge is to define the appropriate dosage, the possible toxicity and to further stabilize the siRNA molecule for in vivo application. Related to the later issue, we are working with other groups (for example, Sirna and Sequitur) to evaluate their chemically modified siRNA duplex in the mouse model.

Some questions and plans:

While writing the SBIR application, and doing our preliminary in vitro and in vivo experiments, we have been asking ourselves that: 1) Whether the ocular NV inhibition was the result of siRNA-mediated gene knockdown? 2) What percentage of siRNA has been delivered to the targeted tissue with TargeTran™? 3) What is the appropriate dosage for PolyTran™-mediated local siRNA delivery? 4) What is the appropriate siRNA for either local or systemic deliveries and 5) What is the duration of siRNA function in mice? To address these issues, we designed the following experiments:

1. siRNA-mediated knockdown of target genes *in vitro*:

Purpose: to evaluate siRNA-mediated knockdown of endogenous targets (mVEGFA, mVEGFR1, mVEGFR2) and/or exogenous (mVEGFA) in tissue culture with RT-PCR (RS-PCR) at mRNA level, and Western blot or ELISA at protein level.

Tissue culture: For better fit our study goal, we made some changes in the choice of cell lines to use in our in vitro experiments.

RAW264.7 gamma NO (-): ATCC, CRL-2278. The mouse monocyte/macrophage cell line, to be used for CpG-induced expression of endogenous mVEGFA. There are three major reasons to choose this cell line. 1) Since it's murine epithelial origin, the CpG or HSV vDNA induction of this cell line mimicks the conditions in the mice NV model; 2) it has much higher transfection efficiency (50-57%) when transfected using LipofectAmine 2000, than the originally chosen J744A.1 (1%); 3) this cell line fails to produce the inflammatory nitric oxide (NO) upon IFN- γ treatment alone, thus is ideal for RNAi study in vitro, where IFN-like reactions are to be avoided.

SVR: ATCC CRL-2280. Mouse endothelial cell line, a candidate cell host for expression of exogenous mVEGF receptors.

Other cell lines: may also be used when needed. For example, in some in vitro study to demonstrate RNAi-mediated inhibition of exogenous pro-angiogenic proteins.

Detailed Procedure: Using RAW264.7 NO (-) cell line, for knockdown of mVEGF, and SVR cell line, for that of endogenous mVEGFR1.

Step 1: CpG induction (adding 0.3 ug into 35-mm wells of tissue culture), or HSV infection. Step 2: Transfect cells siRNA with LipofectAmine 2000.

Knockdown of endogenous mVEGFA in tissue culture

Group	SiRNA	Amount (ug) per 35-mm well		
1	No	0	0	0
2	LacZ(1+2)	0.1	0.5	1.0
3	mVEGFA(1+2)	0.1	0.5	1.0

Step 3: Isolate RNA for RS-PCR or protein for Western blot (or ELISA). Step 4: Doing RT-PCR or Western blot to detect the expression of mVEGFA at RNA or protein level, respectively.

The knockdown of the endogenous expression of this target will be tested the similar way except that siRNAs targeting mVEGFR1 are used to replace the mVEGFA in above table (cells can be infected/induced or not, by HSV or CpG), and measured by RS-PCR or Western blot.

For detection of the knockdown of exogenous mVEGFA, the procedure is basically the same as above, except using plasmid expressing mVEGFA to co-transfect 293 or other cell lines, with correspondent siRNA duplexes.

2. RNAi mediated knockdown of target genes in mouse eyes:

Description: Doing RS-PCR or Western blot to detect the knockdown of target genes by the siRNAs.

Procedure: Freeze the eyeballs, livers, and lungs from tested mice one day after micro-pocketing or infection (see *in vivo* test below), isolate RNA for RS-PCR or isolate protein for Western blot.

3. RNAi-mediated inhibition of corneal NV *in vivo*:

Description:

Experiments are to be performed in the following ways respectively:

- a. Doing RNAi experiment on CpG-induced (or HSV induced) NV as experiments we did with TargeTran™ or PolyTran™ (see experiment design below) except the addition of saline as delivery control (using saline to replace the polymers), to see if we can see NV inhibition only in groups with the use of polymers but not in saline delivery group. This experiment will indicate the efficiency of either of the polymer systems we used.
- b. Induce NV by CpG or HSV. Then, systemically or locally deliver FITC-labeled siRNA (siEGFP or siLacZ) into mice (using TargeTran™ or PolyTran™, respectively), and exam the limbal and corneal area of mice eyes under fluorescence microscope. The fluorescence seen in eye tissues will indicate the delivery of siRNA in corneal NV area. Tissues as liver or lung will also be sampled the same way, but negative of fluorescence detection will support the tissue specificity delivery of siRNA by our polymers. This assay has its limitation, that is, we will not know whether the siRNAs degrade or not while the fluorescence is seen. However, combine this observation with the functional experiment (measuring NV inhibition by), will give some idea about the sustaining of functional siRNAs in eye tissues.

The siRNA dosage, number of mice, and the mice groups are listed in following table.

Reduction of NV by siRNAs *in vivo*

Local Delivery (PolyTran™)				Systemic Delivery (TargeTran™)			
Group	siRNA	# mice	siRNA Dosage (μ g/eye)	Group	siRNA	# mice	siRNA Dosage (μ g/mouse)
1	mVEGF-A(a+b)	10	10	1	mVEGF-A(a+b)	10	40
2	LacZ(a+b)	10	10	2	LacZ(a+b)	10	40
3	Saline (no polymer) simVEGFA(a+b)	10	10	3	Saline (no polymer) simVEGFA(a+b)	10	40
4	FITC-siLucZ	2	10	4	FITC- siLacZ	2	40

5	Saline (no polymer) FITC- siLacZ	2	10	5	Saline (no polymer) FITC- siLacZ	2	40
Subtotal		34				34	

Notes:

1. Every mouse's eyes will be CpG-treated or HSV infected six hours before siRNA delivery.
2. Half of the mice in group 1, 2, and 3 (5 mice/group) will be sacrificed one day after NV induction. Eyeballs will be collected isolation of RNA and/or protein, to be used for RT-PCR and Western blot, respectively.
3. Eyes, livers, and lungs of mice in group 4 and 5 are subjected to fluorescence microscopy detection one day after siRNA delivery, for tissue specificity of delivery (by TargeTranTM and/or PolyTranTM).
4. For Groups 1,2,3 (each has 5 mice left) NV areas are measured on Day 4 post NV-induction.

Figures:

RNAi inhibition of NV in Mice Eyes (Exp. 02, Local delivery, Day 4)

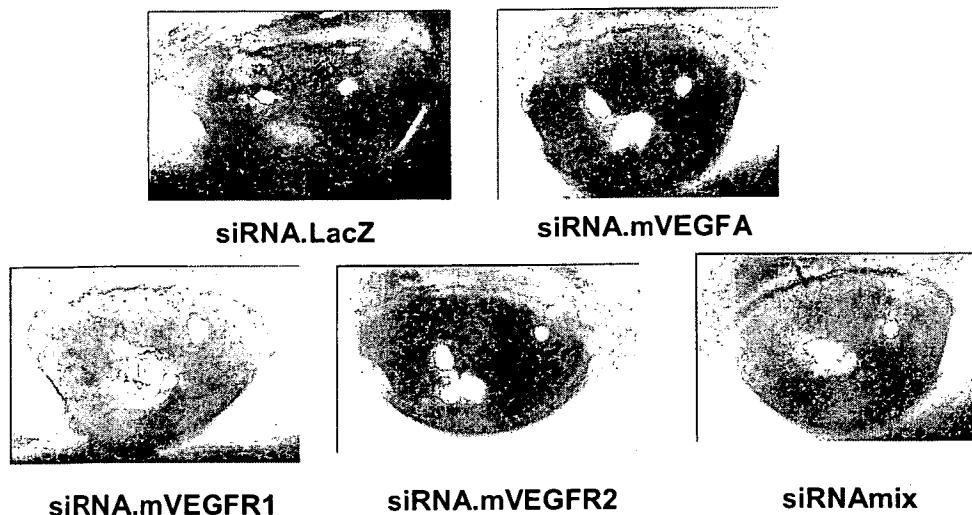


Figure 1. Local delivery of siRNA inhibits NV in Mouse Eyes Hydron pellet containing 1 μ g CpG ODN was implanted into mouse corneal micropocket. Ten μ g of siRNA duplexes targeting each gene with PolyTranTM in 2 μ l solution were delivered subconjunctivally. The siRNA/PolyTranTM was mixed by ratio of 1:8 by weight. Photos were taken 4 days post pocketing for comparison of NV areas. Four eyes were used for each cohort group. siRNAmix means 10 μ g of equally mixed siRNA duplexes targeting all three genes was used.

RNAi inhibition of NV in Mice Eyes

(Exp. 02, Systemic delivery, Day 4)

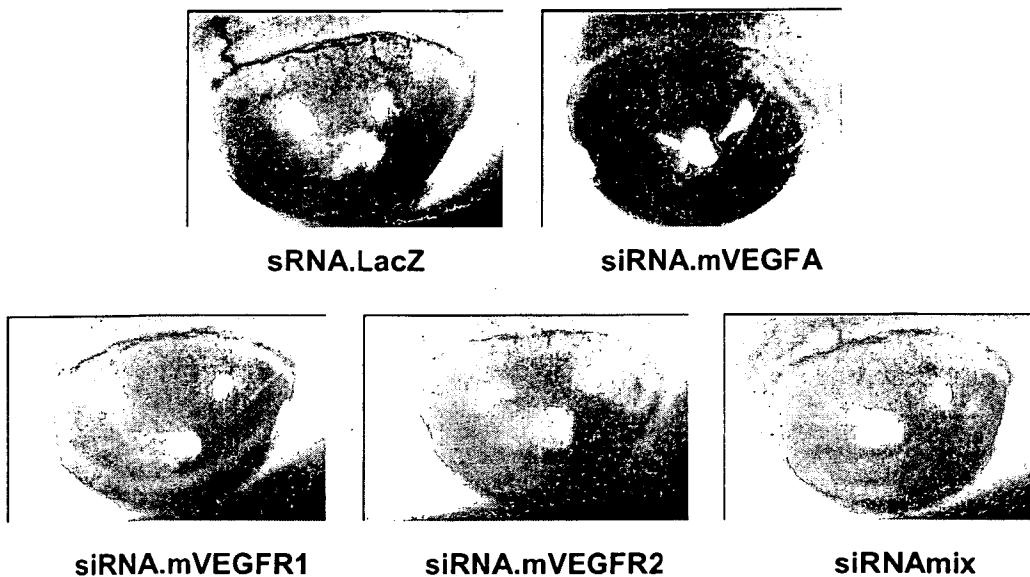


Figure 2. Systemic delivery of siRNA inhibits NV in Mouse Eyes (Day 4) CpG ODN was implanted into micropocket as described in Fig. 1. siRNA duplexes were mixed with TargeTran™ at 1:2 ratio (N/P=2, +/-, nitrogen/phosphate), and administrated by tail vein injection with dosage of 40 µg per mouse 6 hours post CpG induction. Photos were taken 4 days later. Five mice per group were tested. Names of the tested siRNAs are indicated the same as Figure 1.

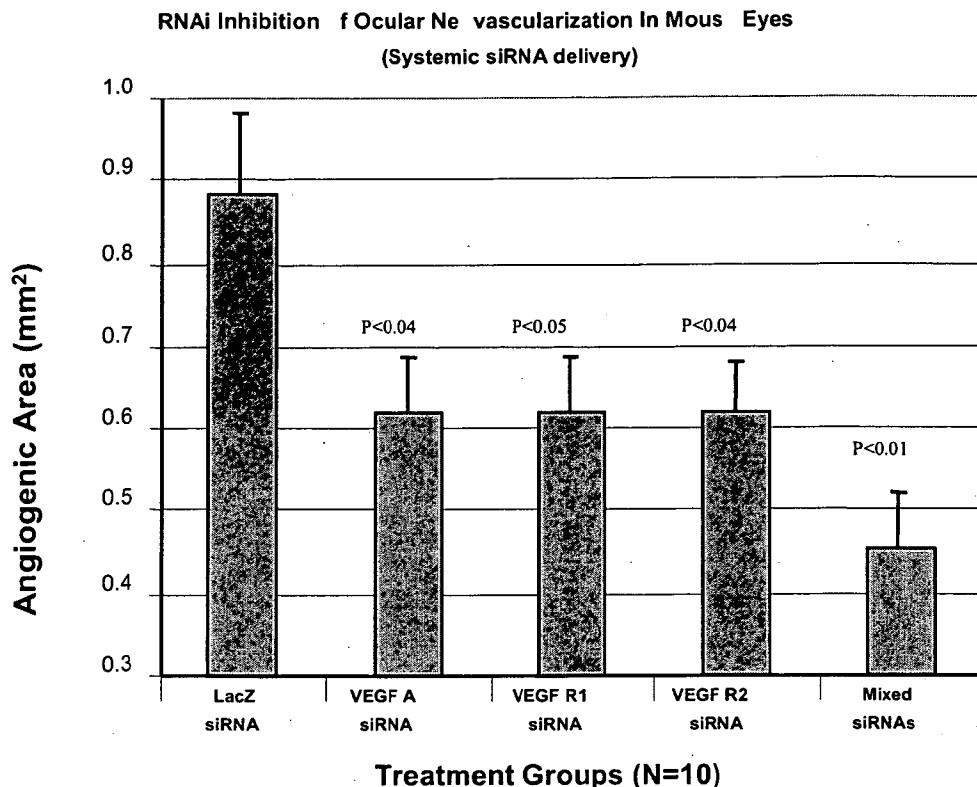


Figure 3. Systemic delivery of siRNA inhibits NV in Mouse Eyes (Day 4) The same experiment as described in Figure 2. Angiogenic area was calculated 4 days post micropocketing, according to the formula for an ellipses. $A = [(clock\ hours) \times 0.4 \times (vessel\ length\ in\ mm) \times \pi]/2$.

RNAi inhibition of NV in Mice Eyes (Exp. 02, Systemic delivery, Day 7)

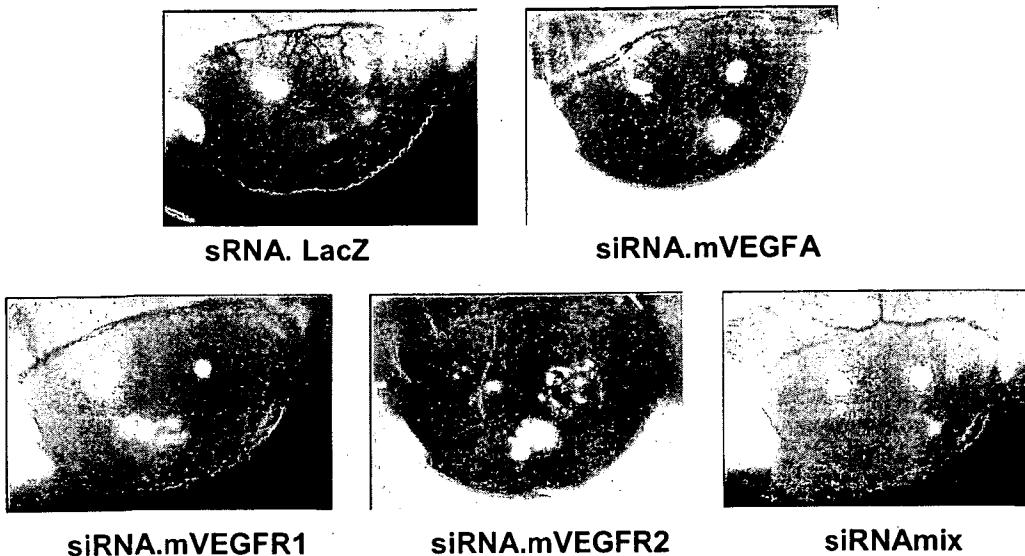


Figure 4. Systemic delivery of siRNA inhibits NV in Mouse Eyes (Day 7) CpG
ODN was implanted into micropocket as described in Fig. 1. siRNA duplexes were
mixed with TargeTran™ at 1:2 ratio (N/P=2, +/-, nitrogen/phosphate), and administrated
by tail vein injection with dosage of 40 μ g per mouse 6 hours post CpG induction.
Photos were taken 7 days later. Five mice per group were tested. Names of the tested
siRNAs are indicated the same as Figure 1.

Fig. 5 Silences Angiogenesis Factors

$$\text{Angiogenic Area} = [(\text{Hours} \times 0.4 \times (\text{Vessel Length in mm}) \times \pi)/2]$$

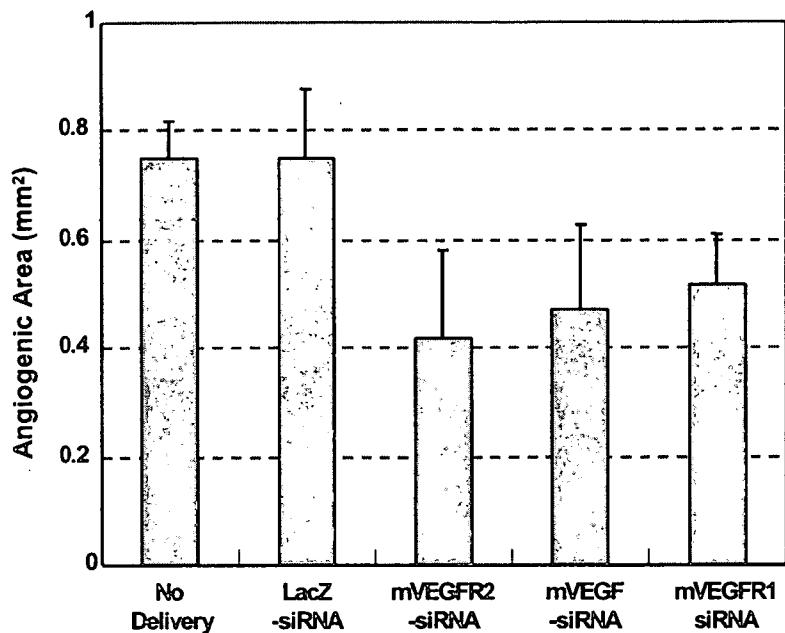


Fig. 6 Inhibits Neo-Vascularization

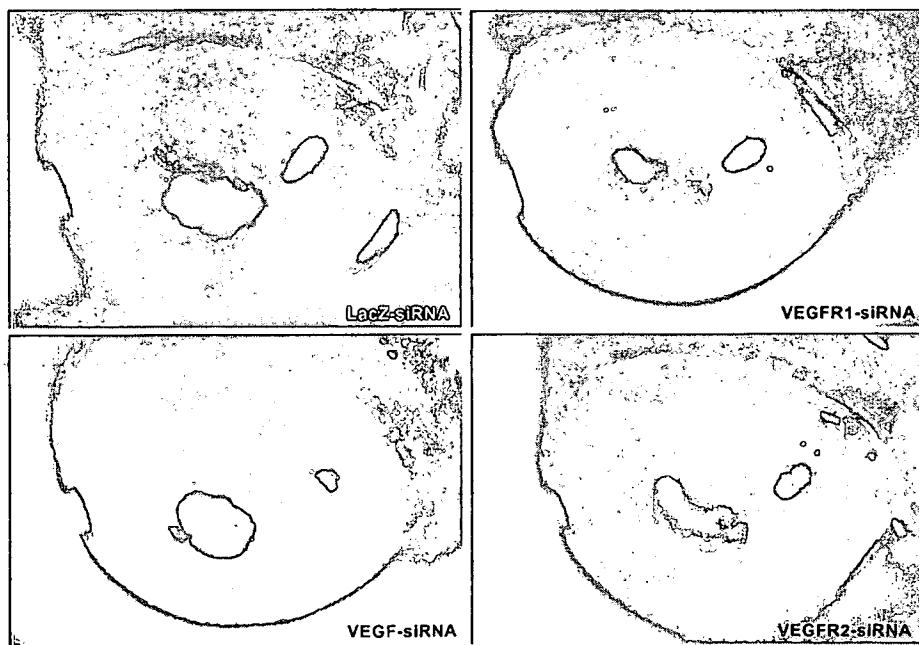
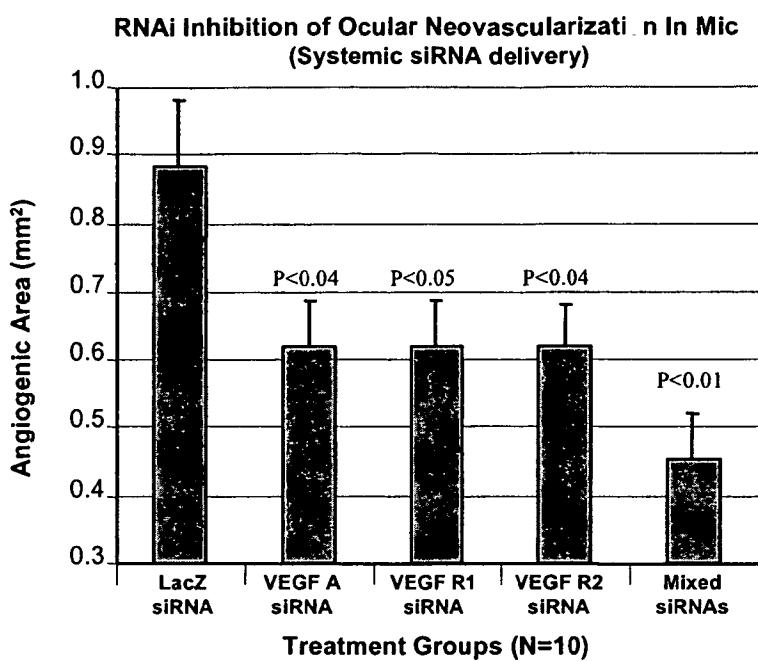


Fig. 7 **TargeTran™-siRNA Therapy**



Application Data Sheet

Application Information

Application number:: Unassigned
Filing Date:: February 5, 2004
Application Type:: Provisional
Subject Matter::
Suggested classification::
Suggested Group Art Unit::
CD-ROM or CD-R?:: No
Number of CD disks::
Number of copies of CDs::
Sequence submission?:: No
Computer Readable Form (CRF)?:: No
Number of copies of CRF::
Title:: RNAi Therapeutics for Treatment of Eye
Neovascularization Diseases
Attorney Docket Number:: 38147-0026
Request for Early Publication?:: No
Request for Non-Publication?:: No
Suggested Drawing Figure::
Total Drawing Sheets:: 6
Small Entity?:: Yes
Latin name::
Variety denomination name::
Petition included?::
Petition Type::
Licensed US Govt. Agency::
Contractor Grant Numbers::
Secrecy Order in Parent Appl.?:: No

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- OR -

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Application::	Continuity Type::	Parent Application::	Parent Filing Date::

Foreign Priority Information

Country::	Application number::	Filing Date::	Priority Claimed::